

PRENATAL DIAGNOSIS BASED ON FETAL BLOOD OBTAINED AT FETOSCOPY

by

Eric Cordesius



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PRENATAL DIAGNOSIS OF SEVERE ALPHA₁-ANTITRYPSIN DEFICIENCY
BY ANALYSIS OF FETAL BLOOD OBTAINED AT FETOSCOPY

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SUMMARY

Two women had each borne a child who had α_1 -antitrypsin (α_1 AT) deficiency and who developed cirrhosis of the liver. In subsequent pregnancies the women asked for prenatal diagnosis. Samples of blood from the two fetuses were obtained at fetoscopy. Samples from five normal fetuses aborted by hysterotomy served as controls. Of the two fetuses at risk, one had an α_1 AT concentration within the Pi MZ range and an electrofocusing pattern indicating a heterozygous Pi MZ phenotype. This infant appeared normal at birth. The other fetus at risk had a severely decreased concentration of α_1 AT and a pattern of a homozygous Pi ZZ phenotype. Analysis of blood from the abortus confirmed these findings and thus the diagnosis of severe α_1 AT deficiency.



SPECULATION

Most of the α_1 AT in amniotic fluid is derived from the mother. It therefore appears that the only possible way of making a prenatal diagnosis of α_1 AT deficiency is by examination of blood from the fetus. One fetus examined in the present study had an abnormal Z-pattern. This abnormality may indicate a disturbance of fetal liver function already in utero.

INTRODUCTION

Most of the α_1 AT in amniotic fluid is derived from the mother (1,4) and is therefore inappropriate for prenatal diagnosis of α_1 AT deficiency. The only possibility for making such a diagnosis seems to be examination of blood from the fetus.

The development of techniques for obtaining fetal blood in utero have made prenatal diagnosis possible in fetuses at risk for α_1 AT deficiency. We report here the prenatal evaluation of two fetuses at risk for the severe form of this disease.

MATERIAL AND METHODS

Clinical material

Case 1. The Pi MZ father and Pi MZ mother had two healthy children; a Pi ZZ girl had died of cirrhosis of the liver at the age of three years. In the 22nd week of a subsequent pregnancy, fetal blood was obtained at fetoscopy for prenatal diagnosis.

Case 2. The Pi MZ father and Pi MZ mother had a Pi ZZ girl. The girl developed severe cirrhosis of the liver. At the age of seven years, an unsuccessful attempt to construct a porta-caval shunt was done. In the 20th week of a subsequent pregnancy, fetal blood was sampled at fetoscopy.

Controls

Samples of maternal blood and amniotic fluid were collected from five women admitted for therapeutic abortion in the 16th to 20th week of gestation. After the pregnancy had been terminated by hysterotomy, fetal blood was sampled by free flow from the pendant umbilical cord.

Fetal blood sampling

In case 1, fetoscopy-guided puncture of placental vessels was done without any attempt at cannulation, and samples of the stream of blood that issued into the amniotic fluid were aspirated (2). In case 2, pure fetal blood was obtained by cannulation of a large vessel near the

insertion of the umbilical cord into the placenta (17). In each woman, a sample of maternal blood was obtained from a cubital vein, and, immediately before fetoscope, a sample of amniotic fluid was obtained by fine-needle amnioncentesis.

The blood samples were immediately transferred to EDTA-tubes.

Identification of fetal blood

The proportion between fetal and maternal red blood cells was estimated by the Kleihauer technique (7). In addition, the relation between HbA and HbF was assessed by electrofocusing at pH 3-10 (5).

Evaluation of the dilution factor

The ratio of fetal blood to amniotic fluid in the fetoscopic samples was calculated from the hematocrit, assuming that the mean hematocrit in pure fetal blood at midpregnancy is 37% (3, 14).

Protein quantitation

The concentration of α_1 AT was estimated by the electroimmunoassay technique (10).

Electrofocusing technique

The fetoscopic samples were reeduced with cysteine to a final concentration of 30 μ mol/ml to achieve a clear microheterogeneous

pattern and less background staining. The freshly prepared cysteine solution used consisted of 0.3 M cysteine-HCl, 2 M glycine and 0.01 M EDTA, pH adjusted to 7.4 with NaOH. A volume of 10 μ l of the cysteine solution was mixed with 90 μ l of plasma or serum and stored over night in the refrigerator.

Ready-prepared thin-layer polyacrylamide gel (PAG) plates with Ampholine at pH 4-5 (LKB, Bromma, Sweden) were used together with LKB 2117 Multiphore apparatus cooled to 15°C with an external cryostat (15). Electrode strips were moistened with 1.0 M glycine at the cathode end 1.0 M phosphoric acid at the anode end.

The gel was pre-run for 1 h with an LKB 2103 power supply of 500 V, 50 mA and 20 W maximum. Samples of 15 μ l cysteine-reduced plasma were applied to filter papers (10 x 15 mm) on the gel. They were placed along the cathodal edge of the gel about 10 mm from the cathodal strip and about 1 mm between papers to ensure mixing of the samples. For the analysis the power supply was set at 1400 V, 50 mA, 20 W for 1 h, and thereafter increased to 30 W for another 2 h. Total effective running time, including pre-run, 4 h.

After completion of the focusing, the gel was placed in fixative consisting of trichloroacetic acid (10 %) and sulfosalicylic acid (5% w/v) for 30-60 min. Staining was performed in 0.4% (w/v) of Coomassie R 250 in the destaining solution consisting of methanol, acetic acid and distilled water (35:10:55, v/v) for 45 min. The microheterogeneous pattern of α_1 AT was clearly visible after another 60 min in the destaining solution.

Immunofixation technique

Immunofixation was carried on the surface of the electrofocusing gel after completion of the run using the "immunoprint technique" (16). Reduced samples were diluted with 0.05 M glycine to a final α_1 AT concentration of approximately 0.08 g/l suggesting that the mean α_1 AT concentration in a normal population is 1.35 g/l (6).

15 μ l of the dilution was subjected to electrofocusing as earlier described. A cellulose acetate membrane (Sepraphore III, Gelman) was soaked in α_1 AT antisera (IgG-fraction, DAKO, Denmark) diluted with a 0.1 M phosphate buffer pH 7.4 (1:4). After completion of the run, the antibody-impregnated cellulose acetate membrane was placed over the expected α_1 AT area on the polyacrylamide gel for 1 h. The membrane was soaked in saline over night and stained for 5 min in 0.2% Coomassie R⁻250 in the methanolacetic acid water mixture.

RESULTS

Controls

Samples obtained at five hysterotomies (therapeutic abortions of Pi MM fetuses) in the 16th to 20th week of gestation showed that the mean concentration of α_1 AT in amniotic fluid was 0.16 g/l (range 0.14-0.24); in fetal plasma, 0.73 g/l (range 0.70-0.80); and in maternal plasma, 1.84 g/l (range 1.60-2.10), assuming 1.35 g/l as the mean concentration in adult plasma (6).

Diagnostic cases

On examination by the Kleihauer technique all the fetoscopic samples were found to contain > 95% fetal red blood cells. Electrofocusing run on hemolysates from isolated red cells showed that the proportions between the two HbF and the minor HbA fractions were normal in all the samples, indicating fetal origin.

Case 1

In all samples obtained from case 1, the fetal blood was diluted with amniotic fluid. In the sample with the highest hematocrit, that is, the less diluted sample, it was 15%; in this sample the concentration of α_1 AT was 0.38 g/l. In pure amniotic fluid, the concentration of α_1 AT was 0.24 g/l. Calculated from the hematocrit, the ratio of fetal blood to amniotic fluid was about 2:3 in the

fetoscopic sample. From this ratio, the fetal plasma concentration of α_1 AT was calculated as 0.6 g/l. This value is consistent with a fetal contribution of α_1 AT, at least from a Pi MZ subject. (A Pi ZZ fetus may have about 10% of the normal fetal plasma α_1 AT concentration, that is, about 0.1 g/l, and a Pi MZ fetus, 70%, that is, about 0.5 g/l (11)). Electrofocusing of this sample showed that the density of the major bands was greater than that in pure amniotic fluid, indicating a fetal contribution of M protein. The identity of the α_1 AT fractions was further demonstrated by immunofixation (Fig. 1). Thus, the α_1 AT concentration and the electrofocusing pattern showed that the fetus had at least one Pi^M gene and, accordingly, was not predisposed to α_1 AT deficiency.

Pregnancy was uncomplicated and terminated in the spontaneous birth of a baby girl in the 41st week of gestation. Analysis of plasma from the newborn infant showed a heterozygous Pi MZ pattern, and the concentration of α_1 AT was 0.95 g/l. Thus, as predicted prenatally, the severe form of α_1 AT deficiency could be excluded.

Case 2

The only sample obtained in case 2 was collected by cannulation of a large vessel near the insertion of the cord. Hematocrit 39%. α_1 AT concentration in the fetal plasma was 0.06 g/l; in amniotic fluid, 0.15 g/l. The fetal plasma had a Pi ZZ pattern both at electrofocusing (Fig. 2) and immunofixation. The Pi MZ pattern of amniotic fluid was identical with that of the controls and with that of case 1. The findings indicated an α_1 AT deficient fetus. The pregnancy was terminated and examination of blood from the abortus confirmed the diagnosis of severe α_1 AT deficiency.

Examination of the liver from the abortus showed signs of cholestasis. No inclusion bodies were visible.

Discussion

Recently a German group (18) used only amniotic fluid proteins to evaluate the phenotype of a fetus at risk for α_1 AT deficiency. But it had been shown previously that amniotic fluid proteins are mainly of maternal origin (1,4). Our results clearly show that the fetal α_1 AT phenotype is fully expressed in fetal plasma in midpregnancy and that the amniotic fluid proteins cannot be used for prenatal diagnosis of α_1 AT deficiency.

We have thus shown that the phenotype and the concentration of α_1 AT can be determined in a fetoscopic sample of fetal blood even when diluted with amniotic fluid. But pure fetal blood is better, as shown in case 2. Immunofixation of good quality is a prerequisite since the actual area on the electrofocusing pattern can sometimes be covered by other smearing proteins, which render interpretation more difficult. Also heparin, when used as an anticoagulant instead of EDTA, can have the same effect on the results. Why the Z-allele contribution was weaker in the MZ amniotic fluid is unknown.

In case 2 the Z-pattern showed an abnormality (Fig. 2), suggesting a disturbance of liver function of the fetus, which was confirmed by the cholestasis observed at autopsy.

The liver and the yolk sac produce α_1 AT, but the contribution from the yolk sac is no longer detectable after the 11th week of gestation (1). At about 10 weeks the α_1 AT concentration is 70% of the adult level, which is reached at 26 weeks (1). At term the mean serum level is 150% in the fetus compared with 200% in the mother (9). A materno-

fetal transfer of α_1 AT has to our knowledge not been measured directly. However, judging from comparison of genetic types of transferrin (12), Gc-globulin (4), orosomucoid (4), and albumin in amniotic fluid and maternal serum, most amniotic fluid proteins are of maternal origin. Our findings showed the same for α_1 AT. However, with the methods used, it was not possible to detect any 5-10% contribution from the fetus to the amniotic fluid proteins. These groups of proteins, similar to albumin in molecular size, behave in a way differing from that of fetal alpha-fetoprotein, which contributes to the protein concentration in amniotic fluid.

In Scandinavia, 1 in 1,500 individuals has α_1 AT deficiency Pi ZZ (19). In a prospective study of 120 Swedish Pi ZZ infants, 11% developed neonatal cholestasis and another 7% had other clinical symptoms of liver disease (19). At birth, about one out of every two infants with neonatal cholestasis was small for gestational age, which may indicate that the infant had the disease already in utero (19). It has been estimated that the risk of a child of Pi MZ parents having congenital liver disease progressing to cirrhosis is 1-2% (13, 19). Judging from our experience, certain Pi ZZ children with a family history of α_1 AT deficiency and severe liver disease may run an even greater risk (19). Emphysema in early life is rare. Our present knowledge of a α_1 AT deficiency Pi ZZ and lung disease in adults suggests that severe early emphysema may be prevented to a considerable extent by avoidance of exposure to tobacco smoke and polluted air (8).

Thus, prenatal examination for α_1 AT deficiency is indicated mainly in fetuses in women who have already borne a Pi ZZ child with severe liver disease. It may also be indicated in those rare families with a Pi Z- or Pi-- child, who run a considerable risk of severe emphysema in early life (20).

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LEGENDS

Fig. 1

The immunofixation pattern after electrofocusing at pH 4-5 of plasma samples from case 1 and from two normal controls. A, plasma from a MM control. B, plasma from a ZZ control. C, amniotic fluid from the MZ mother. D, plasma from the MZ mother. E, mixture of fetal blood and amniotic fluid from the fetus. Z-band contribution in the amniotic fluid is relatively weak (C). The Z-band in the mixture of fetal blood and amniotic fluid (E) is very faint, compared with that of pure amniotic fluid (C), as less protein material was applied.

Fig. 2

The electrofocusing pattern of samples from case 2 after direct staining. A, Pure fetal blood obtained from the abortus. The fetal blood obtained at fetoscopy (B) had an atypical Z-pattern with extra fractions between the two major bands. B, Pure fetal blood obtained at fetoscopy. C, Plasma from the MZ mother.

Fig. 1

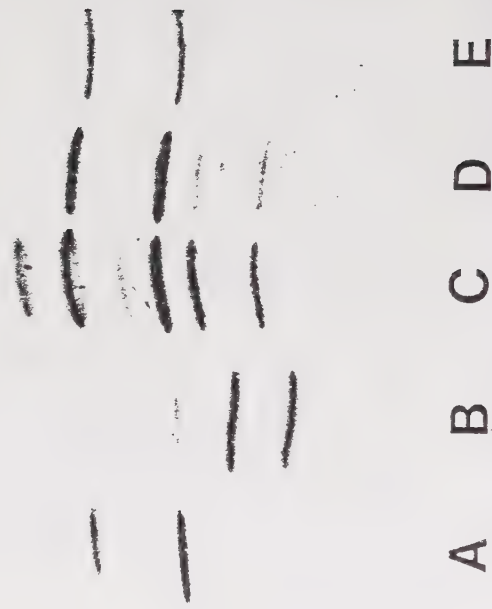
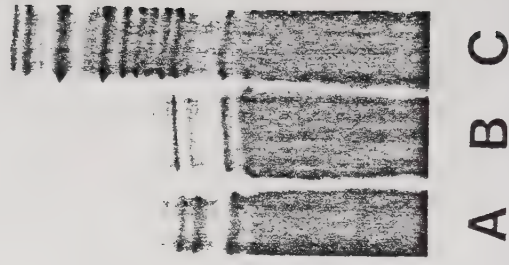


Fig. 2



**PRENATAL DIAGNOSIS BASED ON FETAL BLOOD
OBTAINED AT FETOSCOPY**

AKADEMISK AVHANDLING

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Abstract Sampling of fetal blood in utero was first reported in 1974 (Hobbins & Mahoney, N Engl J Med 290:1065-8). On that occasion the fetoscope was inserted through the abdominal wall. Under direct visual control, the blood-drawing needle was guided forward into a vessel on the surface of the placenta. The technique was found to involve two major problems. First, when the entire placenta was attached to the anterior wall it was often inaccessible. Secondly, the blood-drawing needle usually penetrated the entire vessel and passed into the intervillous space, i.e., into the maternal circulation. The sample obtained was consequently contaminated with maternal blood. We attacked these problems in the following ways. In cases of anterior placenta, the fetoscope was inserted through the anterior vaginal fornix. To avoid contamination of the sample with maternal blood, the placental vessel was punctured without any attempt at cannulation, and a sample of the stream of blood that issued into the amniotic fluid was then aspirated. This technique was first studied in twenty-six women admitted for therapeutic abortion in the second trimester. Ultrasound examination had shown the placenta to be located anteriorly. The insertion of the fetoscope was uncomplicated in all the twenty-six women. In twenty-one of the women, the samples obtained by placental vessel puncture contained only fetal red blood cells; in four, the samples were contaminated with maternal blood; and in one, the instrument was too short to reach the placenta. Later, this technique was used for diagnostic purposes. Although the microsamples of fetal blood obtained were heavily diluted with amniotic fluid, it was for the first time possible prenatally to diagnose or exclude the following inherited disorders. (In cases of posterior placenta, the transabdominal route of fetoscopy was used.) 1) Hemophilia B 2) von Willebrand's disease 3) α_1-antitrypsin deficiency It was also possible for the first time to exclude Mb Down within 72 hours by analysis of such a fetoscopic sample. The methods used for determining the anti-hemophilic factor IX and the fetal karyotype in mixtures of fetal blood and amniotic fluid were developed in our laboratories. These methods are described in detail.			
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Cover photograph

Distal end of the fetoscope with the blood-drawing needle puncturing a vessel on the surface of the placenta. Photo (ex utero after hysterotomy in the 18th week of gestation): Ingemar Nilsson, University Hospital, Lund. (From Gustavii, Cordesius, et al. 1979.)

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ABSTRACT

Sampling of fetal blood in utero was first reported in 1974 (Hobbins & Mahoney, N Engl J Med 290:1065-8). On that occasion the fetoscope was inserted through the abdominal wall. Under direct visual control, the blood-drawing needle was guided forward into a vessel on the surface of the placenta. The technique was found to involve two major problems. First, when the entire placenta was attached to the anterior wall it was often inaccessible. Secondly, the blood-drawing needle usually penetrated the entire vessel and passed into the intervillous space, i.e., into the maternal circulation. The sample obtained was consequently contaminated with maternal blood. We attacked these problems in the following ways. In cases of anterior placenta, the fetoscope was inserted through the anterior vaginal fornix. To avoid contamination of the sample with maternal blood, the placental vessel was punctured without any attempt at cannulation, and a sample of the stream of blood that issued into the amniotic fluid was then aspirated. The technique was first studied in twenty-six women admitted for therapeutic abortion in the second trimester. Ultrasound examination had shown the placenta to be located anteriorly. The insertion of the fetoscope

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- 1) Hemophilia B
- 2) von Willebrand's disease
- 3) α_1 -antitrypsin deficiency

It was also possible for the first time to exclude Mb Down within 72 hours by analysis of such a fetoscopic sample.

The methods used for determining the anti-hemophilic factor IX and the fetal karyotype in mixtures of fetal blood and amniotic fluid were developed in our laboratories. These methods are described in detail.

This thesis is based on the following papers:

I Gustavii B, Cordesius E

Transvaginal fetoscopy in anterior placentas.

Acta Obstet Gynecol Scand 1980; 59:231-2.

II Cordesius E, Gustavii B, Mitelman F

Prenatal chromosomal analysis of fetal blood
obtained at fetoscopy.

Br Med J 1980; 1:1107.

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Lancet 1979; 2:191-2

IV Holmberg L, Gustavii B, Cordesius E,
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Blood 1980; 56:397-

V Jeppsson JO, Cordesius E, Gustavii B, Löfberg L,
Franzén B, Strömberg P, Sveger T

Prenatal diagnosis of severe α_1 -antitrypsin
deficiency by analysis of fetal blood obtained
at fetoscopy.

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The following abbreviations are used in the text:

AFP	Alpha-fetoprotein
EVF.....	Erythrocyte volume fraction (= hematocrit)
Pi	Proteas inhibitor
RBC	Red blood cells
vWD	von Willebrand's disease
VIII:C	Factor VIII clotting activity
VIII:C(Ag)	Factor VIII coagulant antigen
VIII:R:Ag	Factor VIII-related antigen

INTRODUCTION

How to sample fetal blood in utero was first reported by Hobbins and Mahoney in 1974. In their study (done on women to undergo therapeutic abortion) they faced two major problems. First, placentas entirely on the anterior wall of the uterus were often inaccessible. Secondly, the samples obtained were usually contaminated with maternal blood. We have attacked these problems in the present study.

Hobbins and Mahoney's technique in 1974 is briefly described below. Under local anesthesia, an oval cannula (2.2 by 2.7 mm in outer diameter) with a central trocar was inserted through the abdominal wall into the amniotic cavity (Fig. 1). The trocar was removed and an endoscope (Dyonics' Needlescope, 2.2 by 2.2 mm) and a 27-gauge needle were inserted through the cannula. Under direct visual control, the 27-gauge needle was guided forward into a vessel on the surface of the placenta. (The vessels on the surface of the placenta all contain fetal blood.) Negative pressure was applied with a syringe attached to the hub of the 27-gauge needle, and 50 to 500 microliters of blood were aspirated.

Hobbins and Mahoney found that the blood-drawing needle usually penetrated the entire vessel and passed

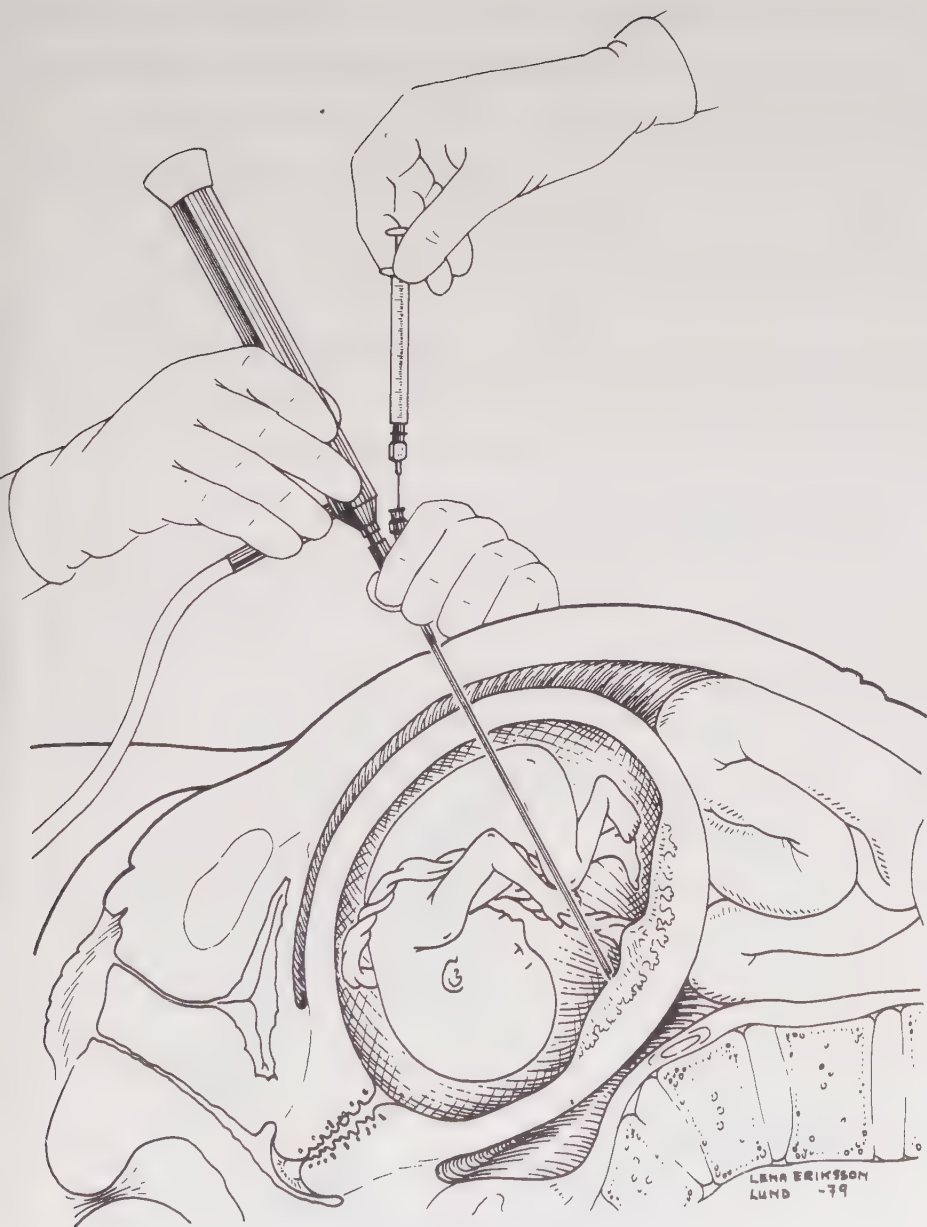


Fig. 1. The fetoscope inserted by the abdominal route.
(From Cordesius et al. 1979.)

into the intervillous space, that is, into the maternal circulation. As a consequence, the sample obtained was mixed with maternal blood. Thus, in seven out of the eight cases studied, the samples were contaminated with maternal blood. Although the diagnosis of hemoglobinopathies, such as sickle cell anemia and thalassemia, can be made on the basis of a mixture of fetal and maternal blood (Alter et al. 1976), biochemical diagnoses, such as those of coagulation defects and many enzymopathies, require analysis of fetal plasma uncontaminated with maternal plasma.

In the present study we attacked the above problems in the following ways. When the placenta was attached to the anterior wall, we inserted the fetoscope through the anterior vaginal fornix (Fig. 2). And to avoid contamination with maternal blood in the sample, we did not cannulate the placental vessel, but picked its surface until it was punctured, and then aspirated a sample of the stream of blood that flowed into the amniotic fluid. A short report on this technique was published in 1976 (Gustavii & Cordesius).

After three years' experience with fetoscopy at therapeutic abortions, we started fetoscopy for the purpose of prenatal diagnosis. Although the microsamples of fetal blood obtained with our technique were heavily diluted with amniotic fluid,

they allowed prenatal diagnosis or exclusion, for the first time, of the following inherited disorders:

- 1) Hemophilia 'B'
- 2) von Willebrand's disease
- 3) α_1 -antitrypsin deficiency

It was also possible for the first time to exclude Mb Down within 72 hours by analysis of such a fetoscopic sample.

The methods used for determining the anti-hemophilic factor IX and the fetal karyotype in mixtures of fetal blood and amniotic fluid were developed in our laboratories.

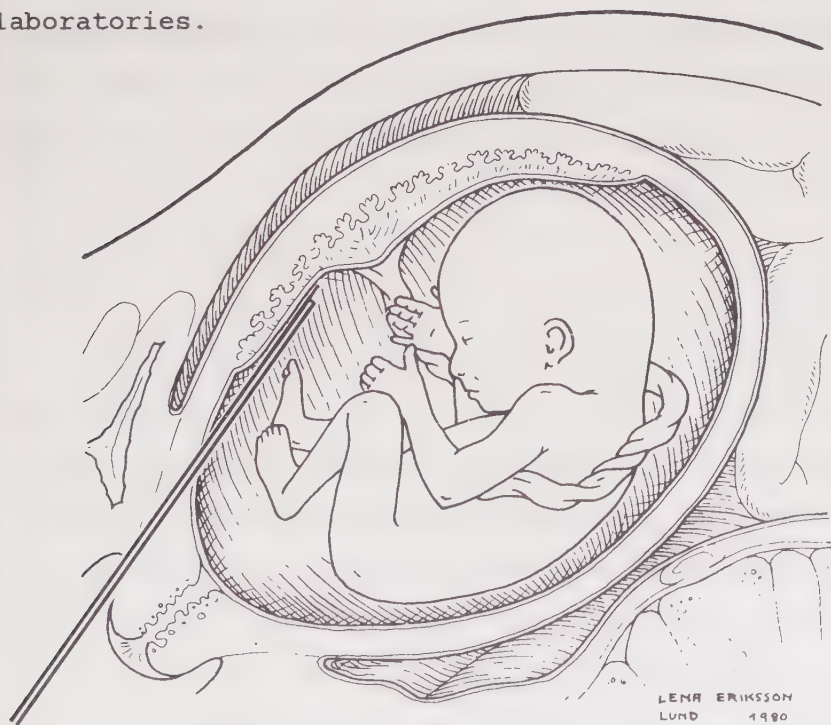


Fig. 2. The fetoscope inserted by the transvaginal route.

MATERIALS AND METHODS

Transvaginal fetoscopy

Methodology study

Twenty-six women admitted for therapeutic abortion in the 16th to 24th week of gestation were selected for this study. Ultrasound examination had shown the placenta to be located anteriorly.

Diagnostic cases

The transvaginal route of fetoscopy was used in five cases for diagnosis; two fetuses were at risk for hemophilia A, one for hemophilia B, one for von Willebrand's disease; in the fifth case, fetoscopy was done for chromosomal studies.

Technique

With the woman in the lithotomy position, the bladder was emptied by catheterization. The vulva and vagina were rinsed with an antiseptic solution. The area where the portio merges with the anterior vaginal fornix was disinfected with iodine and anesthetized. With the cervix held in position with a tenaculum, a sharp trocar and cannula, 2.2 x 2.7 mm in outer diameter, were inserted parallel to the cervix into the amniotic cavity.

Transabdominal fetoscopy

The technique described by Hobbins and Mahoney (1974) was used for fetoscopy by the transabdominal route (Fig. 1).

Collection of fetal blood

After insertion of the cannula with its trocar into the amniotic cavity, the trocar was removed and an endoscope, 1.7 mm in outer diameter ('Needlescope', Dyonics, Woburn, Massachusetts), was inserted through the cannula. Thereafter, a 27-gauge (0.4 mm) blood-drawing needle was inserted through the cannula alongside the endoscope. (The cross section of the cannula is oval.) Under direct visual control, placental vessels were punctured without any attempt at cannulation, and a sample of the stream of blood that issued into the amniotic fluid was aspirated. The proportion between fetal and maternal red blood cells was determined with the Kleihauer staining procedure (Kleihauer et al. 1957).

Chromosomal studies

Methodology study

The fetuses of 13 women admitted for therapeutic abortion in the 16th to 23rd week of gestation were studied. In one of them fetal trisomy 18 had been diagnosed by amniocentesis.

Diagnostic case

The woman had previously borne a child with Down's syndrome. In the 22nd week of a subsequent gestation it became obvious that a second attempt to culture amniotic fluid cells had failed. Since she refused to accept the risk of having another affected child she agreed to prenatal diagnosis by analysis of fetal blood obtained at fetoscopy.

Technique

A fetoscopic sample (2 ml) of blood mixed with amniotic fluid was aspirated and immediately transferred to a glass tube containing 3.5 IU heparin. After centrifugation at 125 g for 10 minutes the supernatant was discarded and the cell button was deposited in a 6-ml Falcon plastic tube containing 1 ml McCoy's 5a medium (Flow Laboratories) with 20% fetal bovine serum and 0.026 ml phytohaemagglutinin

supplemented with streptomycin and benzyl penicillin. After incubation for 65-70 hours at 37°C cell growth was stopped by adding colchicine in a final concentration of 0.125 microgram per milliliter. After hypotonic treatment with potassium chloride 0.075 mmol/l for 10 minutes at room temperature the cells were fixed in three parts methanol and one part acetic acid. Slides were prepared with the standard air-drying technique. The chromosomes were stained with the trypsin-Giemsa banding technique according to Seabright (1971) as slightly modified by Özkinay and Mitelman (1979).

Prenatal exclusion of von Willebrand's disease

von Willebrand's disease was evaluated prenatally in a fetus at risk by analysis of mixtures of blood and amniotic fluid obtained at fetoscopy by the transvaginal route. The mother had previously borne a girl with severe von Willebrand's disease. Where, as in this family, both parents are heterozygous for the von Willebrand's allele(s), the risk of a child having the severe (homozygous) form of this condition is 25%.

Fetal blood was sampled during the 19th week of gestation. Ultrasound scanning showed that the placenta

covered the entire anterior uterine wall. Insertion of the fetoscope (170 mm) through the left abdominal flank lateral to the placental margin failed to reach the amniotic cavity. Two days later the fetoscope was successfully inserted transvaginally. Mixed samples (1 ml) of blood and amniotic fluid were obtained by puncture of the thin vessels at the edge of the lower part of the placenta. The samples were immediately transferred to glass tubes containing 0.1 ml of 3.8% sodium citrate solution and kept at 4°C until analysis, which was done by Dr. Leon Hoyer.

Factor VIII-related antigen (VIII:R:Ag) was determined by an immunoradiometric assay which identifies antigenic determinants that react with heterologous (rabbit) anti-human factor VIII (Hoyer 1972). These determinants are reduced in the plasma of most patients with von Willebrand's disease. Factor VIII coagulant antigen (VIII:C(Ag)) was determined by an immunoradiometric assay (Lazarchick & Hoyer 1978) in which human antibody reacts with antigenic determinants closely associated with factor VIII coagulant activity. These determinants are also reduced in most patients with von Willebrand's disease. Factor VIII coagulant activity was measured by a one-stage assay using hemophilic plasma as substrate (Lazarchick & Hoyer 1978).

Prenatal exclusion of hemophilia B by an immunoradiometric assay of factor IX

Based on homologous antibodies arisen in a hemophilic patient, we developed an immunoradiometric assay of factor IX. Hemophilia B was diagnosed prenatally in one case with the use of this assay.

Hemophiliacs

The immunoradiometric assay of factor IX (described below) was tested on twelve patients with severe hemophilia B and on six patients with moderate or mild hemophilia B.

Controls

Amniotic fluid for factor IX determination was collected from women admitted for therapeutic abortion at 16-20 weeks' gestation. After the pregnancy had been terminated by hysterotomy, the fetal blood was sampled either by catheterization of one of the umbilical arteries or by free flow from the pendant umbilical cord. In addition, factor IX was determined in one sample obtained at fetoscopy of a fetus at risk for hemophilia A but found to be normal. The fetoscopic sample in this case had a volume of 0.9 ml which was made up to 1.1 ml by addition of 0.2 ml citrate solution.

Diagnostic case

The woman was born in 1954. She had recurrent hemarthroses and other bleeding symptoms during childhood. In 1960, female hemophilia B was diagnosed with a factor IX activity that was only 2% of normal (Niléhn & Nilsson 1962). It was first thought that the patient might be a homozygote for the hemophilia B gene. If so, it would mean that any son she might have would be a hemophiliac. During pregnancy in 1977, the woman declined genetic counseling. She delivered a son who turned out to be healthy. It thus became evident that she was a heterozygous carrier of hemophilia B with an unusually low level of factor IX (Holmberg et al. 1978). She became pregnant again, and when fetal sexing showed a male fetus the woman decided to proceed with prenatal diagnosis on fetal blood obtained at fetoscopy.

Fetal blood for factor IX determination was obtained in the 19th week of gestation. Ultrasound scanning showed that the placenta covered the entire anterior uterine wall. The fetoscope was therefore inserted transvaginally. A sample of amniotic fluid was collected through the fetoscope. Two placental vessels were punctured and mixed samples (0.6 - 0.8 ml) of blood and amniotic fluid were aspirated into plastic syringes containing 0.2 ml of

3.8% (0.13 M) trisodium citrate solution. EVF was measured gravimetrically to estimate the dilution factor of the sample, assuming a fetal EVF of 37% (Osaki & Naiman 1972; Holmberg et al. 1974).

Immediately after fetoscopy, the woman was given factor IX concentrate intravenously.

Immunoradiometric assay of factor IX

Factor IX antigen was determined with a new immunoradiometric assay. Serum from a patient with hemophilia B, who had developed an inhibitor to factor IX, was used as a source of antibody. The serum had no detectable antibodies to other plasma proteins, but a low titre (1:1) against HB_sAg (RIA-test). The titre against factor IX was 5000 U/dl (1 U being the amount that neutralizes 1 U of factor IX during incubation for 2 hours at +37°C).

IgG from the serum was isolated with protein A-Sepharose (Pharmacia Fine Chemicals) chromatography. Five ml of serum was applied to a 5 ml column. The column was washed with 0.5 M NaCl and the IgG eluted with 0.1 M glycine-HCl buffer, pH 2.5 and dialyzed against 0.04 M phosphate buffer, 0.1 M NaCl, pH 7.4. 0.25 mg of IgG containing 1 inhibitor unit was labelled with 37 MBq ¹²⁵I with the lactoperoxidase method.

The specific, labelled antibody to factor IX was purified by complexing it with insolubilized factor IX

and elution at acid pH according to the following procedure. 60 mg factor IX concentrate powder (Preonativ, Kabi) was dissolved in 4 ml 0.1 M bicarbonate buffer, 0.5 M NaCl, pH 8.0 and added to 6 ml CH-activated Sepharose 4 B (Pharmacia Fine Chemicals). The coupling was allowed to proceed for 2 hours at room temperature. The supernatant was removed and unreacted groups in the gel were blocked by addition of 0.1 M Tris-HCl buffer, pH 8.0. The gel was then washed until A_{280} in the wash approached zero. The labelled IgG was added to the gel and incubated over night at $+4^{\circ}\text{C}$. The gel was then washed first with phosphate buffer, pH 7.4 and then with 0.1 M Tris-HCl, pH 9.0, followed by 0.1 M acetate, 0.5 M NaCl, pH 4.75. Finally, the specific, labelled antibody was eluted with 0.1 M glycine-HCl buffer, 1% bovine serum albumin, pH 3.0.

The test was performed in the following way: 55 x 11 mm polystyrene tubes (Ellerman tubes, Medart, Sweden) were coated with IgG from the serum by incubation with 0.6 ml of a dilution 1:1000 in 0.1 M carbonate buffer, pH 9.6 for 12 hours at $+4^{\circ}\text{C}$. The tubes were washed 3 times with 1 ml of phosphate-buffered saline, 0.1% bovine serum albumin. The samples to be tested in various dilutions with 0.01 M phosphate buffer, 0.1 M NaCl, 6% BSA, pH 7.4,

and in a total volume of 0.4 ml, were then incubated in the tubes for 12 hours at $+23^{\circ}\text{C}$. The tubes were again washed 3 times and 0.4 ml labelled antibody solution was added. The labelled antibody was diluted 20-40 times with 0.01 M phosphate buffer, 0.1 M NaCl, containing 0.1% BSA, 0.05% normal IgG (Kabi) to about 10,000 cpm/0.4 ml. After reincubation for 12 hours at $+23^{\circ}\text{C}$ and washing 3 times with 1 ml of water the tubes were counted in a gamma scintillator.

The immunoradiometric dose-response curve proved to be nearly linear for normal plasma in dilutions 1/128 - 1/2048 with an amount of bound radioactivity ranging from 25 to 3% (paper IV). The sensitivity limit of the method was thus 0.05 U/dl or even lower. To test the accuracy at very low levels pooled normal plasma was diluted 1:1000 and 1:2000 with 0.01 M phosphate buffer, 0.1 M NaCl, 6% BSA, pH 7.4, and each dilution was tested 20 times. For plasma dilution 1:1000 the mean value was 0.090 ± 0.008 (S.D.) U/dl and for dilution 1:2000 it was 0.039 ± 0.005 (S.D.) U/dl. To test the reproducibility at various levels pooled normal plasma was diluted 1:10, 1:100 and 1:1000 with the same buffer, frozen, and tested on 10 different days. The means and standard deviations were 8.9 ± 0.57 , 0.88 ± 0.04 , and 0.095 ± 0.009 U/dl and the coefficients of variation 6.4%, 4.5% and 9.5% respectively.

Factor IX coagulant activity

Factor IX coagulant activity was determined with a one-stage recalcification assay described by Nilsson (1974).

Prenatal exclusion of α_1 -antitrypsin deficiency

α_1 -antitrypsin deficiency was excluded prenatally in a high-risk fetus by analysis of a mixture of blood and amniotic fluid obtained at fetoscopy. The fetus studied had a 25% risk of the severe (homozygous) form of α_1 -antitrypsin deficiency. The mother had previously a girl with this disease; the girl died in cirrhosis of the liver at the age of three years.

In the 22nd week of pregnancy, a fetoscope was introduced into the amniotic cavity by the transabdominal route. A sample of amniotic fluid was collected through the fetoscope. Mixed samples of blood and amniotic fluid were obtained by puncture of placental vessels. As controls served samples obtained at five hysterotomies (therapeutic abortions of normal fetuses).

The concentration of α_1 -antitrypsin was determined in the samples (Jeppsson et al. 1978). Phenotyping was done by an electrofocusing technique

(Pierce et al. 1976). With this technique, the microheterogenous patterns in serum of the normal Pi MM, the heterogenous Pi MZ, and the deficient homozygote Pi ZZ phenotypes can be shown with certainty (Pierce et al. 1976).

RESULTS AND COMMENTS

Transvaginal fetoscopy

Results

The insertion of the fetoscope was uncomplicated in the 26 women in the methodology study. There were no signs of damage to the urinary bladder; thus, in all women the urine was invariably clear. In one woman, who was in the 24th week of gestation, the instrument (150 mm long) was too short to reach the placenta. In the remaining 25 women, placental vessels were punctured. In one of these women, the samples contained only maternal red cells. In three of the women, 60, 85, and 85% of the cells were of fetal origin in the sample that had the highest percentage of fetal red cells. In the remaining 21, the corresponding figure was > 95%.

Out of five women admitted for prenatal diagnosis, the fetus in two was found to have the disease at risk and pregnancy was terminated. In the remaining three, who thus had unaffected fetuses, two pregnancies went on to term without complications; one woman was delivered at 38 weeks; the other, at 40 weeks. The third woman that had an unaffected fetus had undergone amniocentesis twice before fetoscopy; 6 and 3 weeks previously. In this woman the fetoscopy was followed

by intermittent leakage of amniotic fluid to the vagina. Labour started in the 33rd week. She was delivered of a 2.15 kg. normal girl with a 1 minute Apgar score of 9 and a 5 minute score of 10.

Comment

No early complications of fetoscopy by the transvaginal route were seen in any of the 31 women examined. But the number of continuing pregnancies was too small to admit any conclusions about the risk of abortion or premature delivery with this method. We feel that, until further experience with transvaginal fetoscopy has been gained, the abdominal approach, with its well-documented low frequency of abortion and premature delivery, is the method of choice in those cases of anterior placenta where the instrument can be inserted lateral to the placental margin (Benzie & Pirani 1977; Rodeck & Campbell 1978; Philip et al. 1979). But when an anterior placenta has an extension so broad that the abdominal entry is unfeasible, the transvaginal route may be considered. Technically, the insertion of the instrument through the anterior vaginal fornix is easy, since there is no abdominal wall to penetrate.

Chromosomal studies

Results

Analyzable metaphase plates were obtained in 12 out of the 14 cases (Table I). The fetus of the woman who had borne a child with Down's syndrome had a normal karyotype (46, XY) and at birth was normal, as predicted. The diagnosis in the case of fetal trisomy 18, established by amniocentesis, was verified in the fetoscopy sample. Examination of the fetuses after the abortion showed that sex prediction was correct in all of them.

TABLE I. *Chromosomal analysis in fetoscopy samples**

Fetoscopy case No	Gestational age (weeks)	Amount of blood (μ l)	No of analysable metaphases	Karyotype
1	18	130	2	46,XY
2	16	70	3	46,XY
3	19	< 10	0	—
4	19	400	58	46,XX
5	22	280	> 100	46,XX
6	23	90	73	46,XX
7	19	20	1	47,XY, + 18
8	19	< 10	0	—
9	17	30	7	46,XY
10	18	40	4	46,XY
11	22	50	> 100	46,XX
12	18	280	> 100	46,XX
13	22	130	24	46,XX
14†	22	520	> 100	46,XY

*Mixtures (2 ml) of fetal blood and amniotic fluid. The percentage of fetal red blood cells (Kleihauer technique) was 80-90% in samples in cases 1 and 4; the remaining samples contained > 95% fetal red blood cells.

†Diagnostic fetoscopy in a woman who had borne a child with Down's syndrome.

Comment

The essential step in the procedure seems to be centrifuging the mixed sample before culture. Our first attempts to culture the cells without previous centrifugation usually failed. Pure fetal blood uncontaminated with amniotic fluid (Rodeck & Campbell 1978) would certainly be more suitable for culture than a mixed sample. But sampling pure fetal blood at fetoscopy requires cannulation of the placental vessel with a risk of penetrating the chorionic plate and consequent admixture of maternal blood in the sample. The procedure proved diagnostically reliable in the clinical case. Within three days we could tell the woman that the results of our chromosomal studies were normal. This was later confirmed by the birth of a normal infant.

Although amniocentesis remains the method of choice for prenatal diagnosis of chromosomal abnormalities the procedure described may prove a useful alternative when it is impracticable to wait about three weeks for the results of amniotic fluid cell culture.

Prenatal exclusion of von Willebrand's disease

Results

The first fetoscopic sample contained only maternal

RBC, but three subsequent samples contained > 95% fetal RBC (Kleihauer). Two independent measures were used to calculate the dilution of the fetal plasma by amniotic fluid. The RBC count of the mixture provided one value: a fetal RBC count of 2.5×10^6 per microliter and a fetal haematocrit of 0.37 were assumed. The second value was determined by measuring the alpha-fetoprotein (AFP) content of the mixture and assuming a 2 mg/ml plasma concentration for this 19 week fetus (Gitlin & Boesman 1966). Similar values were obtained for the dilution factors using the two methods; 46 and 52 fold dilution in sample 1; 167 and 185 fold dilution in sample 2; and 43 and 43 and 52 fold dilution in sample 3.

The value for factor VIIIIR:Ag were normal (Table II). Although the prenatal assessment in respect of vWD was evident from the VIIIIR:Ag measurements, we also determined VIII:C(Ag). The values were normal in two samples; the third was too dilute for VIII:C(Ag) measurement. Although mild (heterozygous) vWD could not be ruled out, these data excluded the severe form. Pregnancy continued to term without complications.

Labour started spontaneously in the 38th week, and a boy was delivered by caesarean section because of breech presentation and a borderline pelvis.

Coagulation studies, including VIII:R:Ag, were normal and the infant had no abnormal bleeding. Unfortunately, the child had a complex of skeletal malformations including bilateral femoral hypoplasia and club foot, cleft palate, and micrognathia. This is a recognised pattern of malformations (Daentl et al. 1975). Chromosome studies were normal.

TABLE II—FACTOR VIII CONCENTRATIONS IN FETAL PLASMA OBTAINED BY FETOSCOPY

Sample	Plasma concentration (units/ml)*	
	VIII:R:Ag	VIII:C(Ag)
1	1.09	0.55
2	1.02	. . †
3	0.93	0.27
Normal range ‡	0.50–1.55	0.17–0.94

* Values in fetal plasma were calculated from the data for fetal-blood/amniotic-fluid mixtures using the average of the two estimates of dilution (see text).

† Sample too dilute for assessment of VIII:C(Ag).

‡ Values for 12 normal mid-trimester fetuses.

Comment

The evaluation of fetal plasma for hemophilia depends to a large extent to the ratio of VIII:C(Ag) to VIII:R:Ag in the fetal-blood/amniotic-fluid while in vWD the absolute values of both these antigens are low (Lazarchick & Hoyer 1978). Because the fetoscopy samples were heavily diluted with amniotic fluid, the dilution factor had to be calculated accurately, and we did this using both RBC count and AFP content. This study shows that it is possible to diagnose or exclude severe von Willebrand's disease in the mid-trimester fetus. Although the procedure is certainly not indicated in cases where only one parent has vWD, it may be helpful where both parents have the vWD gene.

Prenatal exclusion of hemophilia B by an immunoradiometric assay of factor IX

Results

Factor IX antigen was measured with the immunoradiometric assay in 24 normals. The mean and standard deviation were 108 ± 27 U/dl. Factor IX activity in the same samples was 95 ± 24 U/dl. The coefficient of correlation between antigen and activity was 0.74 ($p < 0.001$). Eighteen patients with hemophilia B were tested. Of twelve patients with

severe hemophilia B (factor IX activity < 1 U/dl) eleven had a factor IX antigen below 1 U/dl. The twelfth patient had received an infusion of factor IX concentrate 5 days before being tested and had a level of 2.5 U/dl. Among the patients with moderately severe and mild hemophilia B the levels of immunologically detectable factor IX were variable (paper IV).

The concentration of factor IX antigen in plasma from nine non-hemophilic fetuses was 1.8 to 10.2 U/dl. Though in small amounts (0.42 to 1.2 U/dl), factor IX antigen was found to be present in amniotic fluid.

The control fetoscopic sample of the normal fetus had an EVF (hematocrit) of 26%. Factor IX antigen in this sample was 3.4 U/dl. In pure amniotic fluid, factor IX was 1.2 U/dl. Assuming that fetal EVF is 37%, the dilution of the fetal plasma with amniotic fluid and citrate solution was 3:2. The amniotic fluid could therefore contribute at most 0.5 U/dl ($2/5 \times 1.2$ U/dl), and in fact even less (since the citrate alone could account for a dilution of the fetal plasma 3:4, see Material and Methods), to the level of 3.4 U/dl observed in the fetoscopic sample. From these data, the fetal plasma concentration was calculated to be at least 4.8 U/dl.

In the case for diagnosis, the woman herself had a low level (1.9 U/dl) of factor IX antigen in plasma and no detectable factor IX antigen in amniotic fluid (Table III). She was negative for HB_sAg.

The sample obtained at fetoscopy in the case of diagnosis contained > 95% fetal red blood cells. Even in the sample with the highest EVF (19%), no factor IX antigen was detected (Table III). It was not possible to assess factor IX coagulant activity in the fetoscopic samples (see Comment).

The woman decided to have her pregnancy terminated, which was done by hysterotomy under cover of factor IX concentrate. Plasma of the male abortus was collected by catheterisation of one of the umbilical arteries. No factor IX antigen and no factor IX coagulant activity could be detected in the fetal plasma.

Table III. Factor IX in the Diagnostic Case

Sample	Factor IX	Factor IX
	Antigen	Coagulant Activity
	U/dl	U/dl
Maternal plasma	1.9	5
Amniotic fluid	< 0.1	- †
Fetal plasma		
Fetoscopic sample	< 0.2 ‡	- †
Postabortal sample §	< 0.1	< 2

† Activity > 20 U/dl apparently owing to interference by unspecific thromboplastic materials in amniotic fluid.

‡ No demonstrable factor IX antigen in fetal plasma diluted about 1:2 with amniotic fluid and citrate solution. Assay sensitivity was 0.1 U/dl in undiluted samples.

§ The sample was collected by catheterisation of one of the umbilical arteries.

Comment

Prenatal diagnosis of hemophilia offers several difficulties. Fetoscopic samples are often mixed with amniotic fluid. Such samples are not suitable for analysis by conventional coagulant assays, since amniotic fluid contains thromboplastic materials (Courtney & Allington 1972; Phillips & Davidson 1972; Hastwell 1978), which may interfere with these assays. The difficulty can be overcome by the use of immunological methods for factor VIII and factor IX. Sensitive immunoradiometric assays of factor VIII have been developed (Peake & Bloom 1978; Lazarchick & Hoyer 1978; Holmberg et al. 1979) and can be applied to fetoscopic samples obtained for prenatal diagnosis of hemophilia A (Firshein et al. 1979; Cordesius et al. 1979; Mibashan et al. 1979; Philip et al. 1979).

A similar method for factor IX is described in this paper. It is far more sensitive than hitherto available immunological methods for factor IX (Roberts et al. 1968; Ørstavik et al. 1975; Thompson 1977). The accuracy and reproducibility of the method were good even at very low levels of factor IX, which is essential in prenatal examinations, since the factor IX level is normally very low in 16-20 week fetuses. In normal adults there were a significant and strong, although not complete, correlation between factor IX

antigen and coagulant activity. The lack of complete correlation is probably due mainly to an unavoidable variation in factor IX activity determinations. The immunoradiometric method gave somewhat lower values for factor IX antigen in our control fetuses, compared with previously published values for coagulant factor IX activity in fetal plasma (Mibashan et al. 1979; Holmberg et al. 1974), probably because it is not influenced by thromboplastic materials.

However, also immunological assays offer difficulties when used for prenatal diagnosis. Hemophilia B exists in several genetic variants. In one type, so called CRM⁻ (cross-reacting material negative), factor IX antigen is not detectable. In a second type, CRM⁺, the amount of immunologically detectable protein is normal, while factor IX activity may still be very low. In other types, factor IX antigen is lowered either to the same level as, or less than that of, factor IX activity (Roberts et al. 1968; Ørstavik et al. 1975; Thompson 1977; Kasper et al. 1977). The situation in severe hemophilia B may thus be more complicated than in hemophilia A, where the severe form is usually associated with no detectable factor VIII:C antigen (Lazarchick & Hoyer 1978; Holmberg et al. 1979; Peake et al. 1979). Nevertheless, the majority of patients with severe

hemophilia B (factor IX activity < 1 U/dl) should be diagnosable immunologically, since at least 60% of them have very little or no demonstrable factor IX antigen (Ørstavik et al. 1975; Thompson 1977; Kasper et al. 1977). With our immunoradiometric assay for factor IX we have found only one patient among twelve with severe hemophilia B to have factor IX antigen above 1.0 U/dl and this patient had been given a factor IX infusion five days before sampling. In patients with moderately and mild hemophilia B we found the different types described by others. Our method is based on a homologous antibody with a presumably narrow specificity and thus perhaps with a lower capacity than rabbit antibodies (Thompson 1977) to detect abnormal variants of factor IX protein (Denson 1973). Still prenatal diagnosis should not be attempted in an individual case before a known affected member of the family has been evaluated.

Another difficulty in the prenatal diagnosis of hemophilia B is that amniotic fluid contains small amounts of factor IX. This was detected with our immunoradiometric assay. Factor IX occurs in amniotic fluid in a concentration of about 0.5 to 1% of that in the maternal plasma, and its origin is probably almost entirely maternal (Johnson et al. 1974; Sutcliffe et al. 1975). This has to be taken into

account in the calculation of the fetal plasma level of factor IX from the values of the samples obtained at fetoscopy and contaminated with amniotic fluid. In this respect factor IX differs from factor VIII, which does not occur in amniotic fluid (Peake & Bloom 1978).

In our diagnostic case the woman had a low level of factor IX antigen in plasma and was obviously a carrier of a CRM⁻ variant of hemophilia B. She had no demonstrable factor IX antigen in the amniotic fluid, which facilitated the evaluation of the fetoscopic samples consisting of mixtures of fetal blood and amniotic fluid. Assay of these samples for factor IX coagulant activity gave false high values (> 20 U/dl), apparently owing to the presence of unspecific thromboplastic materials in amniotic fluid. Coagulant assays of such samples therefore seem unsuitable for prenatal diagnosis of hemophilia B.

In our patient the conditions for prenatal diagnosis were thus unusually favourable. But we feel that our method may be applicable also to common carriers of hemophilia B provided that the following prerequisites are met: the hemophilia is of the CRM⁻ type, the fetoscopic sample is not too diluted, and factor IX antigen in pure amniotic fluid is determined simultaneously.

Prenatal exclusion of α_1 -antitrypsin deficiency

The control samples obtained at five hysterotomies (therapeutic abortions of normal fetuses) in the 16th to 20th week of gestation showed that the α_1 -antitrypsin concentration was 0.16 g per liter (range, 0.14 to 0.24) in the amniotic fluid, 0.73 g per liter (range, 0.70 to 0.80) in fetal serum, and 1.84 g per liter (range, 1.60 to 2.10) in maternal serum.

In the fetoscopic samples of the case for diagnosis, electrofocusing of hemoglobin showed that the samples contained no maternal blood. All samples were diluted with amniotic fluid. In the sample with the highest hematocrit, that is, the less diluted sample, it was 15%; in this sample the concentration of α_1 -antitrypsin was 0.38 g/l. In pure amniotic fluid, the concentration of α_1 -antitrypsin was 0.24 g/l. Calculated from the hematocrit, the ratio of fetal blood to amniotic fluid was about 2:3 in the fetoscopic sample. From this ratio, the fetal plasma concentration of α_1 -antitrypsin was calculated as 0.6 g/l. This value is consistent with a fetal contribution of α_1 -antitrypsin, at least from a Pi MZ subject. (A Pi ZZ fetus may have about 10% of the normal fetal plasma α_1 -antitrypsin concentration, that is, about 0.1 g/l, and a Pi MZ fetus, 70%, that is, about

0.5 g/l (Laurell & Jeppsson 1975)). Electrofocusing of this sample showed that the density of the major bands was greater than that in pure amniotic fluid, indicating a fetal contribution of M protein. The identity of the α_1 -antitrypsin fractions was further demonstrated by immunofixation (Fig. III). Thus, the α_1 -antitrypsin concentration and the electrofocusing pattern showed that the fetus had at least one Pi^{M} gene and, accordingly, was not predisposed to α_1 -antitrypsin deficiency.

Pregnancy was uncomplicated and terminated in the spontaneous birth of a baby girl in the 41st week of gestation. Analysis of plasma from the newborn infant showed a heterozygous Pi MZ pattern, and the concentration of α_1 -antitrypsin was 0.95 g/l. Thus, as predicted prenatally, the severe form of α_1 -antitrypsin deficiency could be excluded.

We believe that this procedure for prenatal analysis of fetal blood for α_1 -antitrypsin deficiency in certain high-risk families may become reasonably safe and reliable.

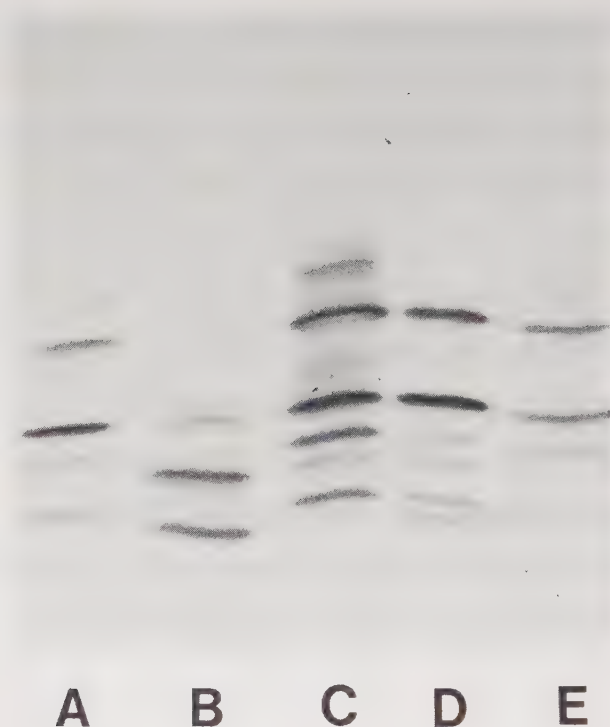


Fig. 3. The immunofixation pattern after electrofocusing at pH 4-5 of plasma samples from the fetus at risk and from two normal controls. A, plasma from a MM control. B, plasma from a ZZ control. C, amniotic fluid from the MZ mother. D, plasma from the MZ mother. E, mixture of fetal blood and amniotic fluid from the fetus. Z-band contribution in the amniotic fluid is relatively weak (C). The Z-band in the mixture of fetal blood and amniotic fluid (E) is very faint, compared with that of pure amniotic fluid (C), as less protein material was applied.

GENERAL SUMMARY

This study of the fetoscopic technique for obtaining fetal blood in utero was done first on women to undergo therapeutic abortion and was used later for diagnostic purposes. Methods for determining the fetal karyotype and the anti-hemophilic factor IX in fetoscopic samples were developed in our laboratories. The results of the present study may be summarized as follows:

- 1) In 31 women, in whom the placenta was attached to the anterior wall, the fetoscope was inserted through the anterior vaginal fornix without any early complications. Three of the women, in whom the examination was done for diagnostic purposes, continued their pregnancies. Two of them went on to term without any complications. In the third, fetoscopy was followed by intermittent leakage of amniotic fluid into the vagina. Labour started in the 33rd week. She was delivered of a 2.15 kg normal girl.
- 2) To avoid contamination with maternal blood in the sample, the placental vessel was punctured without any attempt at cannulation, and then a sample of the stream of blood that issued into the amniotic fluid was aspirated. Usually two or three vessels

were punctured in each case. With this technique samples were obtained from 25 cases of therapeutic abortion. In one case only maternal red blood cells were obtained. In three of the cases, 60, 85, and 85% of the cells were of fetal origin in the sample that had the highest percentage of fetal red cells. In the remaining 21 the corresponding figure was > 95%. Later, this sampling technique was used for diagnostic purposes. The present study included six such cases. In all of them, at least one of the samples obtained in each case contained only fetal blood cells.

- 3) A modified technique for microcultivation of blood was developed for determining the fetal karyotype in fetoscopic samples of mixtures of fetal blood and amniotic fluid. With this technique samples from 13 cases of therapeutic abortion were cultured. Analyzable metaphase plates were obtained in 11 of them. Later, the procedure proved diagnostically reliable in a clinical case where attempts to culture amniotic fluid cells had failed twice. Within three days we could tell the mother that her fetus had normal chromosomes. This was later confirmed by the birth of a normal infant. An essential step in the procedure seems to be centrifugation of the mixture of fetal blood and

amniotic fluid before culture. The first attempts to culture the cells without centrifugation usually failed.

- 4) A sensitive immunoradiometric assay of anti-hemophilic factor IX was developed. The assay was based on homologous antibodies arisen in a hemophilic patient. With this assay, hemophilia B was diagnosed prenatally in one case. Fetoscopic samples of mixtures of fetal blood and amniotic fluid are not suitable for analysis by conventional coagulant assays, since amniotic fluid contains thromboplastic materials that interferes with these assays. Such materials do not influence the immunoradiometric assay.

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